Analysis of antitumor effect of human papillomavirus E6 siRNA-loaded polymeric micelles on human cervical cancer

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ABSTRACT SUMMARY

E6 and E7 oncoproteins of human papillomavirus (HPV) are known to promote tumor growth and malignant transformation. Thus, they are supposed to be suitable targets of nucleic acid drugs for cervical cancer treatment.

In this study, nanosized polymeric micelles with a cyclo-Arg-Gly-Asp (cRGD) peptide on the PEG terminus were developed for efficient delivery of HPV E6 siRNA (E6 siRNA/m).

Ultimately, the systemically administered E6 siRNA/m showed significant antitumor effect on subcutaneous xenograft tumor models which were established by transplanting human cervical cancer cells (Hela) into BALB/c nude mice. This targeted micellar nanomedicine demonstrates the potential utility of systemic siRNA therapies for cervical cancer.

INTRODUCTION

Small interfering RNA (siRNA) therapies have great potential for cancer treatments. E6 and E7 oncoproteins of human papillomavirus (HPV) are known to cause human cervical cancer, because E6 degrades tumor suppressor proteins, such as p53, and also E7 inactivates the performance of pRB tumor suppressor protein. Thus E6 and E7 are supposed to be suitable targets of siRNA therapies against cervical cancers¹,². However, therapeutic siRNA requires a delivery system for transport from the bloodstream into the cytoplasm of cancer cells to perform the function of gene silencing because siRNA is a large watersoluble polynucleotide that cannot easily diffuse across cell membrane. Furthermore, enzymatic activity in blood stream causes rapid degradation of siRNA. Thus, we applied nanosized polymeric micelles that deliver siRNA to solid tumors and elicit a therapeutic effect.

EXPERIMENTAL METHODS

Stable multifunctional micelle having 45 nm in size were prepared by self-assembly of block copolymers of poly (ethylene glycol) and iminothiolane-modified poly (L-lysine) (PEG-b-PLL(IM)) with HPV E6 siRNA (E6 siRNA/m).
The cRGD peptide was incorporated onto the PEG terminus of PEG-b-PLL(IM)³.

In order to evaluate the in vivo antitumor effect of E6 siRNA/m, subcutaneous xenograft models were established by transplanting Hela into BALB/c nude mice. E6 siRNA/m, scramble siRNA/m and 10 mM HEPES buffer as controls were injected (24 µg of siRNA /body) intravenously on day 0, 1, 4, 5, 8 and 9. Tumor volume was measured on day 0, 3, 6, 8, 10 and 12. Tumors were excised from mice on day 12.

In vivo E6 gene silencing effect was evaluated by real time PCR 48 hours after injection.

Fluorescent dye (Alexa647)-conjugated siRNA was used to analyze the accumulation of siRNA in the subcutaneous cervical cancers by IVIS 50 hours after injection. siRNA for luciferase GL3 was used as a control to examine
the effect of siRNA sequence on the tumor accumulation of the micelles.

RESULTS AND DISCUSSION

The antitumor experiment with xenograft mice showed that E6 siRNA/m had 70% tumor growth inhibition effect compared with scramble siRNA/m and buffer control ($p<0.01$), indicating the siRNA sequence-specific antitumor activity of the targeted micelles (Figure 1). Further, the PCR results confirmed that the E6 siRNA/m decreased the expression level of E6 in the tumors (28%, $p<0.05$ for control). Thus, it is demonstrated that the targeted micelles were effective on the cervical cancer treatment through E6 gene silencing.

Biodistribution of E6 siRNA/m was further analyzed by IVIS in comparison with free E6 siRNA as well as GL3 siRNA/m. Figure 2 clearly shows more efficient accumulation of siRNA in the tumors by the targeted micellar formulation, compared with free siRNA. Note that the similar tumor accumulation was observed between E6 siRNA/m and GL3 siRNA/m, indicating that the siRNA sequence does not affect the tumor accumulation of the micellar siRNA.

Figure 1. Tumor volume ratio (tumor volume of day0 =1) in antitumor experiments with xenograft mice models

E6 siRNA/m-injected group showed 70% tumor growth inhibition effect compared with control ($p<0.01$).

Figure 2. Accumulation of fluorescently labelled siRNA in subcutaneous Hela tumors. E6 siRNA/m and GL3 siRNA/m showed enhanced accumulation in the tumors, probably due to the cRGD-based targetability.

CONCLUSION

The cRGD-based targeted micelles achieved the significant antitumor effect for cervical tumors through E6 gene silencing, presumably due to the efficient tumor accumulation of siRNA. Thus, the targeted micelles were demonstrated to have strong potential for systemic siRNA-based therapies for cervical cancer.

REFERENCES

3. Christie, R. J., Matsumoto, Y. et al. ACS nano 2012, 6, (6), 5174-5189.

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